BRIEF NOTE

High Concentration of Blood Glutathione in Dogs with Acute Hemolytic Anemia

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Recently, the author found two canine cases of acute hemolytic anemia associated with an increased erythrocyte glutathione (GSH) concentration, which was 4 to 5 times the mean value in the normal blood.

GSH is a major reducing substance in the erythrocyte and is thought to be important for the membrane integrity of the cell [2]. Genetically determined deficiencies of GSH are known to exist in human beings with hemolytic anemia [3] and in a certain breed of sheep with no apparent hemolytic disorder [5]. Unusually high concentrations of GSH in animal erythrocytes, however, have not been reported. Though Valentine et al. described a few human cases of inherited nonspherocytic hemolytic anemia with an increase in erythrocyte GSH, the cause of the increase was not made clear [6, 7].

In the present cases, the dogs showed severe hemolytic anemia after eating a small quantity of onion in spite of high concentrations of GSH in erythrocytes. This phenomenon cannot be explained with the present knowledge of GSH. It may be necessary to reconsider the present theory concerning the role of GSH in

erythrocyte metabolism.

Routine laboratory investigations were carried out by standard methods. Erythrocyte GSH content was determined by both 5,5-dithiobis-(2-nitrobenzoic acid) method [1] and enzymic method employing glutathione reductase and NADPH [4]. The activity of erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD) and the concentration of adenosine triphosphate (ATP) in erythrocytes were measured by the proprietary preparation, biochemica test-combinations, supplied by the Boehringer Mannheim G.m.b.H., Germany. Hemoglobin electrophoresis was conducted in acrylamide gel.

Case 1. On January 15, 1976, a 6-year-old mongrel female dog showed sudden weakness, depression, and loss of appetite after eating half a boiled onion. The next day she excreted wine-colored urine and was admitted to the Veterinary Hospital of Hokkaido University. Though in good condition, she appeared depressed. Upon investigation she showed the following symptoms: slightly elevated body temperature, extremely pallid mucous membranes, and urine dark brown in color and positive

短報: 赤血球 GSH (還元型グルタチオン) の著増と急性溶血性貧血を呈した犬の 2 例: 前出吉光 (北海道大学獣医学部家畜内科学教室)

Table 1. Reduced glutathione levels (GSH), packed cell volumes (PCV), and reticulocyte count (Retic) of normal and glutathione-excessive dogs

Pedigree	Sex	Age (year)	GSH (mg/100 ml RBC)	PCV (%)	Retic (%)
A	Female	10	340	42.0	4.0
—B	Female	6	305	48.0	3.2
—C	Female	5	133	42.0	3.4
—D	Male	5	85	51.0	1.0
—E	Male	4	100	47.0	4.4
Normal dogs	Mean $\pm$ S.D. (Range)		67 $\pm$ 4.7 (50-89)	45 $\pm$ 3.6 (36-53)	0.32 $\pm$ 0.17 (0.0-1.0)

Remarks.

A: Dam; B: Patient; and C, D and E: Half siblings.

The GSH was determined by DTNB method [1], PCV by microhematocrit, and Retic by new-methylene-blue staining. Each normal value is expressed with the mean $\pm$ standard deviation for samples from 23 normal adult dogs.

for benzidine reaction.

Laboratory test revealed the presence of acute hemolysis. Packed cell volume (PCV) was 23%, erythrocyte count 2,800,000 per mm³, reticulocyte count 21%, and leucocyte count 25,000 per mm³. The differential leucocyte count presented band neutrophils, 1%; mature neutrophils, 74%; lymphocytes, 13%; monocytes, 10%, and eosinophils, 2%. Erythrocyte osmotic fragility test showed normal. No Heinz bodies were observed in wet blood films. Stained blood films showed moderate anisocytosis and severe polychromatophilia.

Blood urea nitrogen (BUN), serum glutamate oxalacetate transaminase (GOT), serum glutamate pyruvate transaminase (GPT), and serum lactate dehydrogenase (LDH) were normal. Erythrocyte G-6-PD activity was in a high normal range. Erythrocyte ATP level was normal. Direct antiglobulin test was negative. Hemoglobin electrophoresis was also normal. Heinz body formation test was positive. Erythrocyte GSH content showed a four-

fold increase, as compared with a reticulocyte-rich blood sample from dog with another type of hemolytic anemia.

One month later blood examinations were carried out again. The PCV, erythrocyte and leucocyte counts were normal. Reticulocyte count was 3.2%, although its normal value was 0.32 $\pm$ 0.17%. Erythrocyte GSH content was 305 mg per 100 ml of erythrocytes, its normal value being 67 $\pm$ 4.7 mg.

Comparative examination was done on blood samples collected from her dam and siblings which were free from similar blood dyscrasia. The erythrocyte GSH content of the dam showed a marked increase, while that of the siblings showed high normal or elevated concentrations. Neither active hemolytic syndrome nor anemia was present, except moderate reticulocytosis, in either of them (Table 1).

Case 2. On July 13, 1976; a male Japanese indigenous dog 3 years old was presented with the complaint of poor appetite and sudden excretion of dark red-colored

urine. The dog had eaten a small amount of onion the day before. On arrival the rectal temperature was 39.7°C and mucous membranes were pallid. There were no signs of vomiting and diarrhea. The urine showed an intensive benzidine reaction and was dark brown in color.

Laboratory tests revealed the following results. PCV was 32%, erythrocyte count 5,250,000 per mm³, reticulocyte count 0.7%, and leucocyte count 26,000 per mm³. The differential leucocyte count exhibited band neutrophils, 9%; mature neutrophils, 74.5%; lymphocytes, 12.5%; and monocytes, 4%.

The following tests at that time were all normal or negative: erythrocyte osmotic fragility, BUN, GOT, GPT, erythrocyte G-6-PD, erythrocyte ATP, Heinz bodies in wet blood films, direct antiglobulin test, and hemoglobin electrophoresis. Heinz body formation test was positive. Erythrocyte GSH content was 277 mg per 100 ml of erythrocytes. After one month, blood examinations were carried out again. Erythrocyte GSH content was 230 mg. The other hematological tests were all normal. No family studies were done on this dog.

In the present studies, it is unclear why the dogs showed acute hemolytic anemia after eating onion, in spite of a high concentration of erythrocyte GSH, and why the GSH content of one dog's (Case 1) dam

and siblings increased so markedly without any evident sign of hematologic disorders. Valentine et al. reported some human cases of hemolytic anemia with high red cell ATP and GSH [6, 7]. They suggested that ribosephosphate pyrophosphokinase deficiency might have been a causative factor of the anemia in those cases.

In the present cases, it is conjectured that the dogs may have had erythrocytes deficient of some specific enzymes, the activity of which may be related to the essential metabolism of erythrocyte glutathione. If the cause of the phenomenon is clarified, it will undoubtedly be possible to unravel some of the more complex problems concerning the current investigation of erythrocyte metabolism.

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